Abstract

Accumulation of mitochondrial DNA (mtDNA) deletions in single cells is commonly observed in many tissues during normal ageing and age-related pathologies. One striking example are dopaminergic neurons of the substantia nigra pars compacta (SNc), which accumulate mtDNA deletions to levels potentially compromising cell survival. Since loss of these neurons is a pathological hallmark of Parkinson's disease (PD), and increased loads of mtDNA deletions have been found in PD patients, their causative role in this pathology was postulated. Also, it is still unclear why other dopaminergic neuron populations, such as those from the ventral tegmental area (VTA), are spared from neuronal death. Additionally, the molecular mechanisms involved in the generation and/or expansion of mtDNA deletions over time have not yet been elucidated. This thesis was initiated to shed light on some of these intriguing phenomena.

Through combined *in vitro* and *in vivo* approaches, and using newly developed methods for the detection of mtDNA deletions, dopamine metabolism was identified as a major contributor to mtDNA deletion accumulation in dopaminergic neurons. Indeed, as in humans, age-related accumulation of mtDNA deletions was more prominent in the SNc of mice, and even higher in adrenal gland, which also synthesises dopamine. Also, neuromelanin, discussed to play a role in the generation of mtDNA deletions, can be excluded as being causative, since mouse nigral neurons are devoid of the black pigment. Further *in vitro* experiments in dopaminergic neuron-like human SH-SY5Y cells support the detrimental role of dopamine on mtDNA integrity. Direct dopamine treatment or enhanced dopamine metabolism after overexpression of key enzymes both induced mtDNA deletions in terminally differentiated cells, while proliferating cells were rather spared. Moreover, inhibiting the uptake of dopamine attenuated its deleterious effect. The newly generated mtDNA deletions were spanning the whole genome, with the exception of indispensable regulatory regions, and were similar to those observed in humans. The mechanisms leading to mtDNA deletions are likely to involve single- and double-strand breaks, as suggested by southern-blot analysis. However, the selective vulnerability of SNc neurons cannot be explained by the presence of mtDNA deletions alone, since single dopaminergic neurons of the VTA were found to harbour the same load of mtDNA deletions.

In conclusion, the accumulation of mtDNA deletions in dopaminergic neurons is driven by dopamine, probably through continuous *de novo* generation caused by strand-break repair. Even though their exact impact on the selective neurodegeneration in PD has still to be clarified, mtDNA deletions are certainly acting in combination with other SNc neuron-specific factors, ultimately leading to cell death.